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File: USPT

L7: Entry 5 of 5

DOCUMENT-IDENTIFIER: US 5851786 A
TITLE: Product and process to regulate actin polymerization

US Patent No. (1):
5851786

Brief Summary Text (15):
Yet another aspect of the present invention includes a method to treat an animal with a disease including cancer, respiratory distress syndrome, inflammatory bowel disease, Parkinson's disease, Alzheimer's disease and restenosis, the method comprising administering to an animal an effective amount of a therapeutic composition comprising a compound that regulates a biological function including actin polymerization, stress fiber formation or focal adhesion assembly.

Detailed Description Text (37):
Acceptable protocols to contact a cell with a putative regulatory compound in an effective manner include the number of cells per container contacted, the concentration of putative regulatory compound(s) administered to a cell, the incubation time of the putative regulatory compound with the cell, the concentration of ligand and/or intracellular initiator molecules administered to a cell, and the incubation time of the ligand and/or intracellular initiator molecule with the cell. Determination of such protocols can be accomplished by those skilled in the art based on variables such as the size of the container, the volume of liquid in the container, the type of cell being tested and the chemical composition of the putative regulatory compound (i.e., size, charge etc.) being tested.

Detailed Description Text (52):
The present invention also includes the determination as to whether a putative regulatory compound is capable of regulating a biological response in a mammal. Such a method entails administering a putative regulatory compound to an animal, such compound being shown, using an assay of the present invention, to regulate actin polymerization, stress fiber formation and/or focal adhesion assembly in a cell. Such a determination is useful for determining conditions under which a putative regulatory compound can be administered to an animal as a therapeutic composition. Thus, it is within the scope of the present invention that those conditions stated herein for testing a compound in an animal can be used when administering a therapeutic composition of the present invention. In particular, a putative regulatory compound can be administered to an animal to determine if the compound is capable of regulating, for example, an inflammatory response, a response to an infectious agent, an autoimmune response, a metabolic response, a cardiovascular response, an allergic response and/or an abnormal cellular growth response in the animal. Acceptable protocols to administer putative regulatory compounds to test the effectiveness of the compound include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of altering a biological response in an animal when administered one or more times over a suitable time period (e.g., from minutes to days or weeks). Preferably, a dose comprises from about 1 nanogram of the compound per kilogram of body weight (ng/kg) to about 1 gram of compound per kilogram of body weight (gm/kg), more preferably 100 ng/kg to about 100 milligrams/kilogram (mg/kg), and even more preferably from about 10 micrograms of compound per kilogram of body weight to about 10 mg/kg. Modes of administration can include, but are not limited to, subcutaneous, rectally, intradermal, intravenous, nasal, oral, transdermal and intramuscular routes. A putative regulatory compound can be combined with other components such as a pharmaceutically acceptable excipient and/or a carrier, prior to administration to an animal. Examples of such excipients include water, saline,

Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, m- or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Carriers are typically compounds that increase the half-life of a compound in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, oils, esters, and glycols. Preferred controlled release formulations are capable of slowly releasing a composition of the present invention into an animal. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release vehicles of the present invention include liquids that, upon administration to an animal, form a solid or a gel in situ. Preferred controlled release vehicles are biodegradable (i.e., bioerodible).

Detailed Description Text (53):
In another aspect of the present invention, the present invention includes conducting a toxicity test on an animal to determine the toxicity of a putative regulatory compound. Toxicity tests for putative regulatory compounds can be performed, for example, on animals after a putative regulatory compound has been determined to have an effect at the cellular level on signal transduction, such as the regulation of cellular inflammatory responses. Such toxicity tests are within the skill of the art, and generally involve testing the toxicity of a compound in vivo or in vitro. A suitable method for testing the toxicity of a putative regulatory compound in vivo can involve scientifically controlled administration of the putative regulatory compound to a number of animals and a period of observance in which the effects of the compound on various aspects of the animal's biological functions (e.g., occurrence of tissue damage, functioning of organs and death) are noted. Suitable methods for testing the toxicity of a putative regulatory compound in vitro can involve scientifically controlled administration of the putative regulatory compound to a cell and subsequent measurement of cell function, cytotoxicity, or cell death. Cell function can be measured by any one of a wide range of assays which will be apparent to one of skill in the art, several of which are herein disclosed (e.g., tyrosine phosphorylation, calcium mobilization and phosphoinositide assays). Methods to measure cytotoxicity are well known in the art and include measurement of the ability to reduce chromogenic substrates such as the tetrazolium-based MTT or sulphorhodamine blue, ATP-bioluminescence assays and fluorescence assays, for example using the Fluorescent Green Protein, among many other readily available assays (see, for example, Bellamy, Drugs 44(5):690-708, 1992, which is incorporated herein by reference in its entirety). Methods to measure cell death include, for example, Coomassie blue staining, acridine orange staining, terminal deoxynucelotidyl transferase (TDT) assays for measuring DNA fragmentation, neutral red exclusion, and measuring changes in forward light scattering in a flow cytometer.

Detailed Description Text (60):
Another aspect of the present invention comprises administering to an animal, a therapeutic composition capable of regulating actin polymerization, stress fiber formation and/or focal adhesion assembly. A therapeutic composition of the present invention is particularly useful for preventing or treating diseases involving abnormal growth or the migration of cells from one location in an animal to another. In particular, a therapeutic composition is useful for preventing or treating diseases involving an inflammatory response, an immune response, an allergic response, a neuronal response, an apoptotic response, tumorigenesis, angiogenesis, metastases, hyperplasia or restenosis. Preferably, a therapeutic composition of the present invention is used to prevent or treat a disease, including, but not limited to, cancer, respiratory distress syndrome, inflammatory bowel disease, Parkinson's disease, Alzheimer's disease or restenosis. A therapeutic composition is preferably administered to a cell, including, but not limited to, a smooth muscle cell, an endothelial cell, an epithelial cell, a basophil, a mast cell, an eosinophil, a neutrophil, a macrophage, a B lymphocyte, a T lymphocyte, a dendritic cell, a natural killer cell, a plasma cell, a neuroblast, a stem cell or a cancer cell. A therapeutic composition is more preferably administered to a cell, including, but not limited to, a small cell lung carcinoma, a

non-small cell lung carcinoma comprising overexpressed epidermal growth factor (EGF) receptors (i.e., having more EGF receptors than the number normally found on non-cancer lung cells), a breast cancer cell comprising overexpressed EGF receptors (i.e., having more EGF receptors than the number normally found on mammary cells), a breast cancer cell comprising overexpressed Neu receptors (i.e., having more Neu receptors than the number normally found on mammary cells), a cancer cell comprising an overexpressed growth factor receptor of an established autocrine loop (i.e., having more growth factor receptors than the number normally found on non-cancer cells) or a cancer cell comprising an overexpressed growth factor receptor of an established paracrine loop (i.e., having more growth factor receptors than the number normally found on non-cancer cells).

Detailed Description Text (61):
A variety of therapeutic compositions can be used to perform the regulation method of the present invention. Such therapeutic compositions include those compounds described in detail herein, in particular, compounds identified using a method of the present invention. A therapeutic composition of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include those described in detail above. In order to regulate the formation of actin polymerization, stress fibers and/or the assembly of focal adhesions in a cell, a therapeutic composition of the present invention can be administered in vivo (i.e., in an animal) or ex vivo (i.e., outside of an animal, such as in tissue culture), in an effective manner such that the composition is capable of regulating actin polymerization, stress fiber formation and/or focal adhesion assembly.

Detailed Description Text (62):
An effective administration protocol (i.e., administering a therapeutic composition in an effective manner) comprises suitable dose parameters and modes of administration that result in prevention or treatment of a disease. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular disease. Such methods include, for example, determination of survival rates, side effects (i.e., toxicity) and progression or regression of disease. For example, the effectiveness of dose parameters and modes of administration of a therapeutic composition of the present invention can be determined by assessing response rates. Such response rates refer to the percentage of treated patients in a population of patients that respond with either partial or complete remission.

Detailed Description Text (63):
In accordance with the present invention, a suitable single dose size is a dose that is capable of preventing or treating an animal with a disease when administered one or more times over a suitable time period. Doses can vary depending upon the disease being treated. For example, in the treatment of cancer, a suitable single dose can be dependent upon whether the cancer being treated is a primary tumor or a metastatic form of cancer.

Detailed Description Text (64):
It will be obvious to one of skill in the art that the number of doses administered to an animal is dependent upon the extent of the disease and the response of an individual patient to the treatment. For example, in the case of cancer, a large tumor may require more doses than a smaller tumor. In some cases, however, a patient having a large tumor may require fewer doses than a patient with a smaller tumor, if the patient with the large tumor responds more favorably to the therapeutic composition than the patient with the smaller tumor. Thus, it is within the scope of the present invention that a suitable number of doses, as well as the time periods between administration, includes any number required to cause regression of a disease.

Detailed Description Text (65):
Therapeutic compositions can be administered directly to a cell in vivo or ex vivo or systemically. Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference in its entirety). Oral delivery can be performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a therapeutic composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Detailed Description Text (79):
Two-to-three hr post-nuclear injection, cells were fixed in 3% paraformaldehyde for 10 min. Cells were rinsed in phosphate buffered saline and permeabilized using 0.2% Triton X-100 for 5 min. The fixed and permeabilized cells were then incubated with DMEM/5%, BCS/5% NCS for 15 min. Expression of microinjected expression plasmid was confirmed by immunostaining permeabilized cells for the presence of .beta.-galactosidase protein using the method described above. Stress fiber formation in injected cells was detected by incubating the permeabilized cells in about 0.2 units/milliliter of Rhodamine-phalloidin (Molecular Probes, Eugene, Oreg.) for about 30 minutes at 24.degree. C. Focal adhesion assembly in injected cells was detected by immunostaining permeabilized cells with a mouse monoclonal anti-vinculin antibody (Sigma, St. Louis, Mo.) and a secondary FITC-conjugated sheep anti-mouse (Cappel) antibody using methods standard in the art. Focal adhesion assembly is represented by the localization of vinculin staining at the leading edge of cells. When cells were stained for vinculin a rhodamine-conjugated goat anti-rabbit antibody (Cappel) was used for detection of .beta.-galactosidase. Coverslips were mounted on slides and examined with a Nikon Diaphot TMD microscope with eipfluorescence. Images of cells were captured using the IPLAB Spectrum digital image analysis program (Signal Analytics Co., Vienna, Va.). All experiments were done at least 3-4 times with similar results.

CLAIMS:

40. A method to treat an animal with a disease selected from the group consisting of cancer, respiratory distress syndrome, inflammatory bowel disease, Parkinson's disease, Alzheimer's disease and restenosis, said method comprising administering to an animal an effective amount of a therapeutic composition comprising a compound that regulates a biological function selected from the group consisting of actin polymerization, stress fiber formation or focal adhesion assembly, said compound being capable of penetrating the plasma membrane of a cell in said animal and of inhibiting the ability of a G protein selected from the group consisting of G.sub.12 and G.sub.13 to regulate Rho protein activity in said cell.

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End of Result Set

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L1: Entry 1 of 1 File: USPT Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180597 B1
TITLE: Upregulation of Type III endothelial cell nitric oxide synthase by rho GTPase function inhibitors

US Patent No. (1):
6180597

Detailed Description Text (43):
Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. Lower doses will result from certain forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.